Isolated Ricin B-Chain-mediated Apoptosis in U937 Cells

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We have previously reported that ricin, a toxic lectin that inhibits protein synthesis induced apoptotic cell death. In this study, we have found that isolated ricin CM-B-chain, which has no effect on cellular protein synthesis, induced DNA fragmentation in U937 cells in a dose- and time-dependent manner, albeit it required a longer incubation time and higher concentration than those of holotoxin ricin. Z-Asp-CH2-DCB, a caspase family inhibitor and serine protease inhibitor, 3,4-dichloroisocoumarine (DCI) effectively inhibited the CM-B-chain-mediated DNA fragmentation as well as in ricin. Thus, like ricin, multiple proteases with different substrate specificity may also be involved in the CM-B-chain-mediated apoptotic pathway. Furthermore, BFA inhibited both ricin- and CM-B-chain-mediated DNA fragmentation, suggesting an intracellular vesicle transport system through the Golgi complex may be involved in the apoptotic induction by these proteins as a common feature. On the other hand, cycloheximide (CHA) strongly increased the CM-B-chain-mediated DNA fragmentation, but inhibited ricin-mediated DNA fragmentation. The opposite effects of CHA may reflect the difference in the apoptotic mechanism between ricin and CM-B-chain. In conclusion, our results suggest that ricin-B-chain can induce apoptosis through its lectin activity, but the underlying mechanism may be distinct from that of ricin in which the A-chain contributes profoundly to the apoptotic induction.

Key words: ricin; ricin B-chain; cytotoxicity; lectin; apoptosis

Ricin, an extremely toxic lectin present in castor bean seeds (Ricinus communis), consists of two polypeptide chains (A- and B-chains) linked together via a single disulfide bond. The A-chain is an enzyme that inactivates the 60S ribosomal subunit by catalyzing the N-glycosidic cleavage of a specific adenine residue from the 28S ribosomal RNA, and the B-chain is a lectin that binds to cell surface receptors containing galactose or N-acetylgalactosamine residues.1,2 The intoxication pathway of ricin consists of (i) binding to cell-surface receptors, (ii) receptor-mediated endocytosis and intracellular transport through the vesicular system, (iii) translocation of whole toxin molecules or enzymatically active A-chain across the vesicle membrane to the cytosol, and (iv) enzymatic inactivation of ribosomes resulting in the inhibition of cellular protein synthesis. Aside from the ability of ricin to inhibit protein synthesis, recent studies have demonstrated that ricin can induce programmed cell death or apoptosis.3,4 In fact, it has been observed that incubation of different cell lines with ricin results in cell death associated with typical apoptotic changes such as membrane blebbing, chromatin condensation, and oligonucleosomal DNA fragmentation.5-8 Other protein toxins such as diphtheria toxin and Pseudomonas toxin have also been reported to induce apoptotic cell death, although their exact mechanisms are still unclear.9,8 Since these toxins are all known to inhibit protein synthesis in the target cells following the binding to specific receptors and subsequent internalization through receptor-mediated endocytosis, the apoptotic signal may be triggered during their intoxication processes.

In addition to these protein toxins, recent studies have demonstrated that some lectins such as phytohemagglutinin (PHA), concanavalin A (Con A), wheat germ agglutinin (WGA), Lens culinaris (LCA), and Grifonia simplicifolia 1-B, (GS1B) lectin are cytotoxic and induce apoptosis, even though these lectins do not inhibit protein synthesis.9,10

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Abbreviations: CM-B-chain, carboxymethylated B-chain; BFA, brefeldin A; WGA, wheat germ agglutinin; Con A, concanavalin A; Z-Asp-CH2-DCB, carboxobenzyl-Asp-1-yl-[2,6-dichlorobenzyl]oxy]methylene; Ac-DEVD-CHO, acetyl-Asp-Glu-Val-Ala-Asp-aldehyde; Ac-YVAD-CHO, acetyl-Tyr-Val-Ala-Asp-aldehyde; Ac-VEID-CHO, acetyl-Val-Glu-Ile-Asp-aldehyde; DCl, 3,4-dichloroisocoumarin; MCA, 4-methyl-coumaryl-7-amide; CHA, cycloheximide; FBS, fetal bovine serum; BSA, bovine serum albumin; LDH, lactate dehydrogenase
Consistent with these findings, isolated ricin B-chain has been shown to be cytotoxic against certain cells, while the isolated A-chain was nontoxic at the cellular level. Although the cooperative action of its constituent polypeptide chains (A- and B-chains) is required for ricin-mediated cellular protein synthesis inhibition, it may be possible that the B-chain induces cytotoxic effect through its lectin activity. However, it remains unknown how binding of the ricin B-chain to cell surface carbohydrates results in cytotoxicity. To gain an insight into this point, we investigated whether isolated ricin B-chain can also induce apoptotic cell death. We studied B-chain-mediated inhibition of protein synthesis and the DNA fragmentation on human myeloid leukemia cells (U937), and compared its activities with those of holotoxin ricin.

Materials and Methods

Materials. Ricin was isolated from small castor beans as described by Mise et al. Ricin B-chain was isolated by affinity chromatography on Sepharose 4B, followed by diethylaminomethyl (DEAE)- and carboxymethyl (CM)-cellulose column chromatographies. The isolated B-chain is spontaneously oxidized to form its dimmer in aqueous solution. To prevent the dimer formation we prepared carboxymethylated ricin B-chain. Carboxymethylation of B-chain was done based on the method of Crestfield et al. as described previously. The hemagglutinating activity of carboxymethylated B-chain (CM-B-chain) against human erythrocytes remained, as previously reported. Brefeldin A (BFA) was obtained from Epicentech Technologies (Madison, WI). Wheat germ agglutinin (WGA) and concanavalin A (Con A) were obtained from Sigma Chemical Co. (St. Louis, MO). [3H]Leucine (60 Ci/mmol) was obtained from Du Pont-New England Nuclear Research Products. The protease inhibitors (Ac-DEVD-CHO, Ac-YVAD-CHO, Ac-VEID-CHO, and Z-Asp-CH2-DCB) were obtained from the Peptide Institute, Inc., Osaka, Japan. 3,4-dichloroisocoumarin (DCI) was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA).

Cell culture. A human myeloid leukemia U937 cell line was obtained from the Riken Cell Bank, Tsukuba, Japan. Cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS). Vero (African green monkey kidney) cells were cultured in α-minimal essential medium (α-MEM) with 10% FBS, 10 μg each of adenosine, guanosine, cytidine, and thymidine ml of medium, penicillin (100 μg/ml), and streptomycin (100 μg/ml) as described. These cell lines were maintained in a humidified atmosphere of 5% CO2 and 95% air.

Measurement of protein synthesis inhibition. Cells in 48-well plates (5 × 103 cells/well) in RPMI-1640 medium containing 35 μM BSA were incubated at 37°C with varying concentrations of ricin or CM-B-chain. After 3 h of incubation, the medium was replaced by leucine-free medium containing 1 μCi/ml [3H]leucine by centrifugation, and then the cells were incubated for 45 min at 37°C. The incorporation of [3H]leucine into perchloric acid/phosphotungstic acid-insoluble materials was measured as described previously. The results were expressed as a percentage of the incorporation in control cells incubated without toxin but otherwise treated in the same way.

Cytolytic activity. Cytolytic activity of ricin or CM-B-chain was measured by a lactate dehydrogenase (LDH) release assay in which LDH released from lysed cells was measured by the 2-(p-iiodophenyl)-3-(p-nitropheno)-5-phenyltetrazolium chloride (INT) reduction as described previously. In brief, 2 × 105 cells/well in a 96-well plate in RPMI-1640 medium containing 35 μM BSA were treated with various concentrations of ricin or CM-B-chain for 24 h at 37°C. Following the centrifugation of the plate (300 × g, 10 min), 50 μl of supernatant of each well was subjected to LDH assay.

Measurement of DNA fragmentation. U937 cells (2 × 106 cells/ml) in dishes (35 mm) were treated with various concentrations of ricin or CM-B-chain in RPMI-1640 medium containing 35 μM BSA at 37°C for the indicated periods of time. After removal of the medium by centrifugation (1,500 × g for 10 min), the cell pellets were lysed in 1 ml of cold lysis buffer containing 0.5% Triton X-100, 10 mM Tris, 20 mM EDTA, pH 8.0. Samples were subsequently centrifuged for 28 min at 13,000 × g to separate DNA fragments (supernatant) from intact DNA (pellet). The DNA contents in supernatant and pellet fractions were measured using the diphenylamine reagent. When the effect of BFA on ricin- or CM-B-chain-mediated DNA fragmentation was examined in Vero cells, adherent Vero cells (1 × 106 cells/dish) in α-MEM containing 35 μM BSA were used. DNA fragmentation was expressed as a percentage using the following equation:

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\text{% DNA fragmentation} = (\text{O.D. of supernatant/O.D. of supernatant} + \text{O.D. of pellet}) \times 100
\]

Nuclear staining. U937 cells in RPMI-1640 medium containing 35 μM BSA were treated with ricin or CM-B-chain for 24 h at 37°C. The cells were washed once with PBS, and then cells were stained with Hoechst 33258 (40 μM), and observed by fluorescence microscopy using a microscope (Olympus BX60).
Results

The protein synthesis inhibitory activities and cytolytic activities of ricin and its isolated CM-B-chain in U937 cells

To examine the protein synthesis inhibitory activities of ricin and its isolated CM-B-chain in U937 cells, cells were incubated with various concentrations of each sample for 3 h at 37°C. After removal of the medium by centrifugation, [3H]leucine incorporation into proteins was measured. As shown in Fig. 1, ricin showed strong inhibitory activity and the IC50 was estimated to be 1.65 ng/ml, but no significant effect of CM-B-chain was observed up to 10,000 ng/ml. These results confirm that there is no contamination by traces of intact ricin in the preparation of CM-B-chain. Since CM-B-chain caused strong aggregation of U937 cells with a similar extent of ricin, its binding ability on the cell surface may remain intact (data not shown).

On the other hand, ricin induced cell lysis in a dose-dependent manner after 20 h of incubation, while CM-B-chain showed only a partial cytolytic activity against U937 cells even at very high concentrations (Fig. 1B). Thus, it seems likely that ricin-A-chain is mainly responsible for the intoxication mechanism leading to cell lysis.

Ricin- and CM-B-chain-mediated DNA fragmentation and nuclear morphological changes in U937 cells

One of the most characteristic features of apoptosis is a nuclear change concomitant with DNA fragmentation due to internucleosomal cleavage of chromosomal DNA. In our previous studies, the analysis of DNA extracted from Vero or U937 cells treated with ricin by agarose gel electrophoresis showed that ricin induced the degradation of DNA and formation of DNA ladder. In this study, we analyzed the ability of ricin and CM-B-chain to induce DNA fragmentation in U937 cells by the diphenylamine assay. As shown in Fig. 2, ricin induced DNA fragmentation in a dose-dependent manner, and 10 ng/ml of ricin caused the maximal level of DNA fragmentation. Interestingly, CM-B-chain also induced DNA fragmentation, and its effective concentration was 10,000 ng/ml, at which CM-B-chain had no effect on cellular protein synthesis (Fig. 1). The ability of CM-B-chain to induce DNA fragmentation in U937 cells was much stronger than that of WGA, while Con A had no effect against this cell line even at 100 μg/ml (Fig. 2). Both ricin- and CM-B-chain-mediated DNA fragmentation were significantly abrogated when the specific inhibitory sugar (lactose) was added simultaneously and maintained throughout the incubation.

Fig. 1. Protein Synthesis Inhibitory Activities (A) and Cytolytic Activities (B) of Ricin (○) or CM-B-Chain ( ● ) in U937 Cells.

(A) Cells in 48-well plates (5 × 104 cells/well) were incubated at 37°C with various concentrations of ricin or CM-B-chain in RPMI-1640 medium containing 35 μM BSA. After 3 h of incubation, the medium was replaced by leucine-free medium containing 1 μCi/ml [3H]leucine, and the cells were incubated for 45 min at 37°C for the measurement of protein synthesis. Each point represents an average of triplicate measurements. (B) Cells in 96-well plates (2 × 104 cells/well) were incubated with various concentrations of ricin or CM-B-chain in RPMI-1640 medium containing 35 μM BSA for 24 h at 37°C, and then tested by the LDH release assay as described under Materials and Methods.

Fig. 2. Dose-Response Curves of DNA Fragmentation Induced by Ricin (○), CM-B-Chain ( ● ), WGA (○), and Con A (●) in U937 Cells.

Cells (2 × 104 cells/dish) were incubated at 37°C with various concentrations of each sample in RPMI-1640 medium containing 35 μM BSA. After 24 h of incubation, DNA fragmentation in the treated cells under each condition was assayed with diphenylamine as described under Materials and Methods. Each point represents an average of triplicate measurements.
period (24 h) (data not shown). Thus, the binding of these proteins on the specific cell surface carbohydrates appears to be a initial step to induce apoptosis as might be expected. Furthermore, apoptotic nuclear morphological change was observed in the cells treated with CM-B-chain, which was indistinguishable from that induced by ricin (Fig. 3). These results suggest that CM-B-chain can induce apoptosis without affecting cellular protein synthesis.

For the kinetic studies of the apoptotic induction by ricin and CM-B-chain, we analyzed the time course of reaction for DNA fragmentation. As shown in Fig. 4, 10 ng/ml of ricin required about 6 h of incubation for significant induction of DNA fragmentation. However, CM-B-chain took a longer incubation time to induce DNA fragmentation, and 10,000 ng/ml of CM-B-chain caused DNA fragmentation to a lesser extent as compared to ricin even after 24 h of incubation. Furthermore, similar time courses of DNA fragmentation between CM-B-chain and WGA were observed (Fig. 4). Therefore, these results suggest that CM-B-chain and WGA may share a common apoptotic mechanism distinct from that of ricin in their temporal aspect.

Effects of caspase-related inhibitors and DCI on the DNA fragmentation induced by ricin and CM-B-chain

It has been well documented that caspase family proteases are important in many cells undergoing apoptosis. In addition, our previous studies have suggested that serine proteases may also be involved in ricin-mediated apoptosis. In fact, we found that Z-Asp-CH$_2$-DCB and DCI inhibited ricin-mediated DNA fragmentation, and prevented eventual cell death as examined by the Alamar blue assay. To study the involvement of proteases in the apoptosis caused by CM-B-chain, we examined the effects of Z-Asp-CH$_2$-DCB (aspartate-based caspase family inhibitor), Ac-YVAD-CHO (caspase-1 inhibitor), Ac-DEVD-CHO (caspase-3 inhibitor), Ac-VEID-CHO (caspase-6 inhibitor), and DCI (serine protease inhibitor) on the DNA fragmentation induced by CM-B-chain or ricin in U937 cells. As shown in Fig. 5, Z-Asp-CH$_2$-DCB completely prevented both ricin- and CM-B-chain-mediated DNA fragmentation. However, three caspase-specific tetrapeptide inhibitors (Ac-YVAD-CHO, Ac-DEVD-CHO, and Ac-VEID-CHO) were ineffective. These results suggest that Z-Asp-CH$_2$-DCB-sensitive caspases except for...
caspase-1, -3, and -6 may be involved in the apoptotic signaling pathway of ricin and CM-B-chain as a common feature. Furthermore, DCI also showed strong preventive effect against both ricin and CM-B-chain (Fig. 5).

Effects of cycloheximide (CHA) and Brefeldin A (BFA) on the DNA fragmentation induced by ricin and CM-B-chain

To further characterize the process responsible for the CM-B-chain-induced DNA fragmentation, we tested the effect of CHA, which is known to affect apoptosis caused by a number of compounds including ricin.\textsuperscript{3,5,20,21} As shown in Table 1, 0.1 μg/ml of CHA strongly increased CM-B-chain-mediated DNA fragmentation, while CHA itself did not induce DNA fragmentation even though 0.1 μg/ml of CHA was enough to inhibit cellular protein synthesis. WGA-induced DNA fragmentation was also slightly increased by the addition of CHA rather than inhibition (data not shown). In contrast, CHA inhibited ricin-mediated DNA fragmentation as previously reported.\textsuperscript{5} These results suggest that there are some differences in the apoptotic mechanisms between ricin and CM-B-chain.

Our previous studies demonstrated that BFA, which is known to affect the Golgi complex, blocked the ability of ricin to induce apoptosis as well as its protein synthesis inhibitory activity in Vero cells.\textsuperscript{5} Regarding the cytotoxic effects of ricin and CM-B-chain, basically similar results were obtained in Vero cells and in U937 cells. Namely, lack of protein synthesis inhibitory activity of CM-B-chain was also confirmed in Vero cells, and CM-B-chain caused DNA fragmentation and cell lysis in Vero cells with lesser extent as compared to ricin (Fig. 6). These

![Fig. 5. Effect of Caspase-Related Protease Inhibitors and DCI on the Ricin- or CM-B-Chain-Induced DNA Fragmentation in U937 Cells.](image)

Cells (2 × 10^6 cells/ml) were incubated in the presence of 100 μM of Z-Asp-CH₂-DCB, Ac-DEVD-CHO, Ac-YVAD-CHO, Ac-VEID-CHO, or DCI in RPMI-1640 medium containing 35 μM BSA for 1 h at 37°C, followed by the addition of ricin (10 ng/ml) ( ), or CM-B-chain (10,000 ng/ml) ( ). The cells were incubated for another 24 h at 37°C, and DNA fragmentation in the treated cells under each condition was assayed with diphenylamine as described under Materials and Methods.

![Fig. 6. Protein Synthesis Inhibition (A), Cytolysis (B), and DNA Fragmentation (C) Caused by Ricin and CM-B-Chain in Vero Cells.](image)

(A) Cells grown in 48-well plates (1 × 10^6 cells/well) were incubated with various concentrations of ricin ( ) or CM-B-chain ( ) in α-MEM containing 35 μM BSA for 3 h at 37°C, and protein synthesis was measured by incorporation of [3H]leucine as described in the legend to Fig. 1. (B) Cells (1 × 10^6 cells/dish) were incubated with 10 ng/ml of ricin ( ) or 10,000 ng/ml of CM-B-chain ( ) in α-MEM containing 35 μM BSA for 24 h at 37°C. DNA fragmentation in each batch treated cells was assayed with diphenylamine as described under Materials and Methods. (C) Cells in 96-well plates (3 × 10^4 cells/well) were incubated with 10 ng/ml of ricin ( ) or 10,000 ng/ml of CM-B-chain ( ) in α-MEM containing 35 μM BSA for 24 h at 37°C, and then assayed by LDH release described under Materials and Methods.
results suggest that characteristic cytotoxic features of ricin and CM-B-chain can be reflected in different cell types. Since BFA itself was cytotoxic to U937 cells, we examined its effect on ricin- and CM-B-chain-mediated apoptosis in Vero cells. As shown in Table 1, BFA also showed inhibitory effect on the CM-B-chain-mediated DNA fragmentation. Thus, like ricin, intracellular events such as vesicle trafficking through the Golgi complex may be partly involved in the CM-B-chain-induced DNA fragmentation.

Discussion

Programmed cell death or apoptosis is a specific process of physiological cell death that plays a homeostatic role in the normal growth and development of a multicellular organism during embryogenesis as well as in adult tissues undergoing cell turnover. Apoptosis has been observed in many types of cells after stimulation by a variety of chemical, pharmacological, and physical agents. It is characterized by the morphological and biochemical features including cell shrinkage, membrane blebbing, endonuclease-mediated DNA fragmentation, the involvement of caspase signalling cascades, and mitochondrial proteins and the role of anti-death proteins such as Bcl-2. Recent studies have shown that ricin induces cell lysis and DNA fragmentation in a process reminiscent of apoptosis.

This study demonstrated that the isolated ricin CM-B-chain can induce apoptosis in U937 cells as judged by DNA fragmentation and typical apoptotic nuclear morphological change, which was indistinguishable from that observed in ricin-treated cells (Fig. 2 and Fig. 3). Since CM-B-chain showed no significant inhibition of protein synthesis up to 10,000 ng/ml at which CM-B-chain caused the DNA fragmentation concomitant with nuclear morphological change, its lectin activity may be responsible for the apoptotic induction. This notion may be supported by the findings that phytohaemagglutinin, Con A, WGA, and Lens culinaris lectin induce apoptotic cell death, even though these lectins do not inhibit protein synthesis. Since a kinetic similarity between WGA and CM-B-chain was observed in DNA fragmentation assay, a lectin-specific mechanism of apoptotic induction may exist (Fig. 4). Moreover, Kim et al. have reported that WGA and Griffonia simplicifolia 1-B4 lectin induced morphological changes typical for apoptosis and DNA fragmentation in tumor cells.

However, it remains largely unknown how binding of these lectins including ricin B-chain to cell surface carbohydrates results in cytotoxic or apoptosis. Regarding this point, the study of cytotoxic activity of Griffonia simplicifolia 1-B4 lectin immobilized on agarose beads suggested that the binding of the lectin to the specific receptors is necessary step but is not sufficient for cell lysis, since immobilized lectin failed to trigger apoptotic cell death. They speculated that lectin internalization and probably subsequent intracellular mechanisms are involved in the lectin-mediated apoptosis. The importance of the post-binding mechanisms for lectin-mediated cytotoxicity is also supported by the finding that WGA-resistant tumor cell variants were able to bind WGA similarly to the parental WGA-sensitive cell line.

The intracellular vesicular transport system is presumably involved in the intoxication pathway of several protein toxins. In the case of ricin, it has been demonstrated that internalized ricin is transported to the Golgi apparatus. Several lines of evidence suggest that ricin is further transported into the trans-Golgi network (TGN) or a post-TGN compartment where the translocation of ricin or its A-chain into cytosol may take place. Our recent results demonstrated that BFA, which is known to affect the structure and function of the Golgi apparatus, strongly prevented ricin-induced apoptotic cell death as well as ricin-mediated protein synthesis inhibition.

Similar to ricin, CM-B-chain-induced DNA fragmentation was also inhibited by BFA in Vero cells (Table 1). These results suggest that some apoptotic signals may be triggered during the intracellular processing or trafficking of ricin B-chain beyond endocytosis similar to ricin.

It has been generally accepted that apoptosis is an active process and the proteolytic activation cascade may be involved in the apoptotic signalling pathway. At a later phase, the signal leads to the activation of an endogenous Ca<sup>2+</sup>/Mg<sup>2+</sup>-dependent endonuclease.
that results in DNA fragmentation.\textsuperscript{37} Although the detailed mechanism by which activation of the endonuclease(s) is still unclear; it appears that an essential component is caspases, a family of cysteine proteases.\textsuperscript{38} Increasing lines of evidence suggested that multiple caspases are involved in the same apoptotic cell. Furthermore, our previous results suggested that serine proteases, which may have distinct substrate specificity from caspases, are also implicated in ricin-mediated apoptosis.\textsuperscript{8} Interestingly, Z-Asp-CH$_2$-DCB, a caspase family inhibitor and serine protease inhibitor, DCI effectively inhibited the CM-B-chain-mediated DNA fragmentation as did ricin (Fig. 5). At present, we cannot identify the actual intracellular target of these inhibitors, one can speculate that multiple proteases with different substrate specificity may be responsible for apoptotic induction by ricin and CM-B-chain as a common feature.

In contrast to the similar sensitivity profiles of ricin and CM-B-chain against protease inhibitors, CHA produced opposite effects on these proteins (Table 1), \textit{i.e.}, this reagent increased the CM-B-chain-mediated DNA fragmentation, but inhibited ricin as previously reported.\textsuperscript{5,39} These results may reflect the different apoptotic mechanisms of ricin and CM-B-chain. In agreement with these results, CM-B-chain required a higher concentration to induce DNA fragmentation even after a longer incubation time than ricin (Fig. 4). According to the previous reports, the effective concentrations of Con A and WGA to cause apoptosis are 10–100 $\mu$g/ml, which are similar to the range of CM-B-chain.\textsuperscript{39,40} In fact, our results also demonstrated that WGA required 100 $\mu$g/ml to cause significant DNA fragmentation in U937 cells, and even 100 $\mu$g/ml of Con A had no effect against this cell line (Fig. 2). Thus, it seems likely that these lectins need higher concentrations for apoptosis than the toxic lectin ricin which has the A-chain that protein inhibits protein synthesis. In other words, the highly efficient apoptotic induction by ricin may be due to the presence of the A-chain. However, the biological significance of protein synthesis inhibition in the cells undergoing apoptosis is still controversial. For instance, well-known protein synthesis inhibitors such as CHA and actinomycin D can inhibit glucocorticoid-induced apoptosis in rat thymocytes,\textsuperscript{39} and radiation-induced apoptosis in lymphocytes,\textsuperscript{42} while CHA itself has been shown to cause DNA fragmentation typical of apoptosis in macrophages.\textsuperscript{40} In this study, however, we found that CHA did not induce DNA fragmentation in U937 cells (Table 1). In addition, it has been shown that macrophage adherence prevents ricin-induced apoptosis but has no effect on the inhibition of protein synthesis by ricin.\textsuperscript{42} These findings suggest that ricin-mediated apoptosis is not a simple or straightforward consequence of the inhibition of protein synthesis. Therefore, the protein synthesis inhibition is not necessarily required for all apoptotic systems, and it depends on cell types and apoptotic stimuli. Probably more than one mechanism are involved in the apoptotic process.

Regarding the enzymatic activity of the ricin A-chain, it has been reported that the A-chain has DNA-damaging activity aside from the inhibition of protein synthesis by cleavage of the N-glycosidic bond of a specific adenine of 28S ribosomal RNA.\textsuperscript{43} Thus, it is possible that the A-chain triggers an apoptotic signal through acting on nuclear DNA in target cell. Further studies are required to clarify the exact role of the A-chain in ricin-mediated apoptotic induction.

References


